Progress in the Development of an HIV-1 Vaccine

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Containment of the acquired immunodeficiency syndrome (AIDS) epidemic will require an effective human immunodeficiency virus type 1 (HIV-1) vaccine. Accumulating evidence suggests that such a vaccine must efficiently elicit an HIV-1–specific cytotoxic T lymphocyte (CTL) response. Nonhuman primate models will continue to provide an important tool for assessing the extent of protective immunity induced by various immunization strategies. Although replication-competent AIDS viruses attenuated for pathogenicity by selective gene deletions have provided protective immunity in nonhuman primate models, the long-term safety of such vaccines in human populations is suspect. Inactivated virus and subunit vaccines have elicited neither CTLs nor antibodies capable of neutralizing a wide array of patient HIV-1 isolates. Considerable effort is now being focused on evaluating live vector-based vaccine and plasmid DNA vaccine approaches for preventing HIV-1 infection both in animal model and human studies. Our growing understanding of the biology of HIV-1 and immune responses to this virus will continue to suggest improved vaccination approaches for exploration.

Although the current generation of antiviral drugs represents a major triumph in the battle against AIDS, most HIV-1–infected individuals will never benefit from these therapeutic agents. The spread of the AIDS epidemic is concentrated in regions of the world where insufficient financial resources are available to allow access to these drugs (*1*). Even in wealthy, industrialized nations where antiviral therapeutics are available, poor toleration of the drugs and the emergence of resistant viruses make long-term responses to antiviral therapies far from universal. In fact, replication-competent HIV-1 persists in lymphoid tissue even in individuals who appear to have responded well to antiviral therapy (*2*). Successful containment of the AIDS epidemic will ultimately require an effective vaccination strategy that prevents the spread of HIV-1.

HIV-1 is a uniquely difficult target for vaccine development. Immune correlates of protective immunity against HIV-1 infection remain uncertain. The virus persistently replicates in the infected individual, leading inexorably to disease despite the generation of vigorous humoral and cellular immune responses (*3*). HIV-1 rapidly mutates during infection, resulting in the generation of viruses that can escape immune recognition (*4*). Virus can persist indefinitely as latent proviral DNA, capable of replicating in individuals at a later time (*5*). Finally, because the usual route of transmission of HIV-1 is through mucosal surfaces, mucosal immunity may be required to prevent sexual transmission of the virus.

In this article, I will review our current understanding of HIV-1–specific immunity to define the characteristics of the virus-specific immune responses that a successful vaccine should elicit. I will then describe the nonhuman primate AIDS models used to test vaccination strategies, indicating the strengths and limitations of each model. Finally, I will review the status of the various HIV-1 vaccine strategies that have been tested, describing both the traditional and novel approaches that have been explored.

Immune Responses to HIV-1 in Infected Individuals

The development of a vaccine to prevent infection by HIV-1 must be based on an understanding of both the humoral and cellular immune responses to the virus. Primary patient HIV-1 isolates do not have a highly immunogenic, shared principal neutralizing determinant (*6*). Although high-titer HIV-1 envelope-binding antibodies are sustained in infected individuals throughout the course of infection, these antibodies have very poor neutralizing activity against autologous as well as representative primary patient virus isolates (*7*). Moreover, the emergence of these weak neutralizing antibody responses is detected long after the containment of the early burst of HIV-1 replication during primary infection (*8*). These observations suggest that neutralizing antibodies that arise in response to HIV-1 infection may not be critical in limiting viral replication.

However, this does not mean that a neutralizing antibody response cannot be elicited by a vaccine or that such an immune response might not protect against HIV-1 infection. A small number of human monoclonal antibodies have been generated from infected individuals with potent neutralizing activity against a diversity of primary patient HIV-1 isolates (*9*). It is now clear that antibodies must bind well to the gp120/gp41 complex on the surface of virions and not merely isolated gp120 in order to neutralize the virus (*9*). Further broadly neutralizing anti–HIV-1 envelope antibody specificities may be defined in the near future as the screening assays used for antibody selection take this understanding into consideration.

$CD8^+$ cytotoxic T lymphocytes (CTLs) appear to be important in containing the spread of HIV-1 in infected individuals. Evidence for this protective role comes from a variety of observations. HIV-1–specific CTLs have been found in large numbers in a variety of anatomic compartments in both HIV-1–infected humans and nonhuman primates with comparable infections: in peripheral blood lymphocytes, bronchoalveolar lavage lymphocytes, lymph nodes, spleen, skin, cerebrospinal fluid, and vaginal mucosal tissue (*10*). The replication of HIV-1 in $CD4^+$ lymphocytes can be inhibited by autologous $CD8^+$ CTLs by mechanisms that probably include both lysis and release of chemokines and cytokines (*11*). The early containment of HIV-1 replication in the infected individual coincides temporally with the emergence of a virus-specific CTL response (*12*). Moreover, in those chronically infected with HIV-1, a high-frequency CTL response is correlated with the maintenance of low virus load and a stable clinical status (*13*). These observations suggest that an effective HIV-1 vaccine should stimulate HIV-1–specific CTLs. This presents an important challenge in HIV-1 vaccine development, because most vaccines in common use for preventing other infectious diseases have not needed to induce effector T cells.

Precisely how efficient an HIV-1 vaccine must be in eliciting CTLs remains an open question. Ideally, an immunogen would induce potent and long-lived CTLs in most or all vaccinees against a variety of virus-encoded proteins. However, the rationale for eliciting HIV-1–specific CTLs by vaccination is that preexisting memory CTLs should be expanded to effector CTLs

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in vivo after virus infection more rapidly and in larger numbers than would naïve T cells. Even a vaccine that elicits sporadically detected CTLs could conceivably induce sufficient memory T lymphocyte responses to facilitate a rapid mobilization of effector CTL after virus infection. The extent of vaccine-induced CTL immunity that will be needed to contain an AIDS virus infection will only be defined through empirical studies.

Animal Models for Assessing HIV-1 Vaccine Strategies

Animal models are needed to test the efficacy of different strategies for eliciting protective immunity against HIV-1 infection (Table 1). The only species other than humans that are susceptible to infection by HIV-1 are the great apes. The most carefully studied of these is the HIV-1–infected chimpanzee. Many primary patient HIV-1 isolates maintain only a low level of replication in chimpanzees, with no detectable viral RNA in the plasma of chronically infected animals (*14*). These isolates do not induce disease in this species. A number of vaccine strategies have protected chimpanzees from infection by such poorly replicating HIV-1 isolates (*15*). However, the low levels of virus replication that are initiated by these infections in the chimpanzee do not provide a particularly rigorous test of vaccine-elicited immunity.

A chimpanzee-passaged isolate of HIV-1 has recently been shown to replicate to high levels, induce $CD4^+$ lymphocyte loss, and cause an AIDS-like syndrome in chimpanzees (*16*). A vaccine challenge stock developed from this isolate would provide an important new tool for testing vaccine approaches. Nevertheless, the high cost and scarcity of chimpanzees will continue to limit their utility for studying the diversity of HIV-1 vaccine strategies that must be evaluated.

Table 1. Nonhuman primate models for testing HIV-1 vaccines.

Species and virus	Limitations
Chimpanzees	
HIV-1	Scarcity of animals, expense, limited viral replication and absence of disease when infected with patient isolates
Macaques	
SIV	Differences from HIV-1 in viral sequences and important envelope epitopes
SHIV	Different from HIV-1–induced human disease in kinetics of $CD4^+$ cell loss

HIV-1 is a member of a large family of primate lentiviruses that includes viruses that infect African nonhuman primate species. These African lentivirus isolates, called simian immunodeficiency viruses (SIVs), are not pathogenic in their natural host species (*17*). Some isolates, however, persistently replicate to very high levels and induce an AIDS-like disease when they infect Asian macaque monkeys. Manifestations of this SIV-induced disease include $CD4^+$ lymphocyte loss, immunodeficiency, wasting, infection by a variety of opportunistic pathogens, and lymphomas (*18*). The SIV-infected macaque has been a crucial model for assessing HIV-1 vaccine strategies over the past decade.

Although the SIVs and HIV-1 in general have substantial nucleotide sequence homology, the envelopes of these viruses are quite divergent (*19*). The antigenic and structural differences between these envelopes have limited the utility of the SIV/macaque model for assessing HIV-1 envelope-based vaccine strategies. Recent studies have shown that chimeric viruses can be constructed in the laboratory that express HIV-1 envelopes on an SIV backbone (*20*). Such constructed viruses, referred to as simian/human immunodeficiency viruses (SHIVs), did not at first persistently replicate to high levels or cause disease in macaques. However, in vivo passage of some of these chimeric viruses in macaques has resulted in SHIVs that induce $CD4^+$ lymphocyte loss and death as a result of opportunistic infections (*21*).

There is no universally accepted approach to virus challenge for testing vaccine strategies in nonhuman primates. Large numbers of female chimpanzees and macaques are not available for extensive protection studies involving vaginal mucosal virus challenges. Investigators therefore use intravenous challenges or, to assess protection against mucosal infection, use intrarectal routes of virus inoculation. Immune protection against virus challenges by these routes may not predict the outcome of exposure to virus through heterosexual contact. Although much of HIV-1 transmission is likely to occur through exposure to cell-associated virus, most nonhuman primate virus challenge studies are done with cell-free virus to simplify the issue of challenge virus preservation and quantitation. Finally, nonhuman primate vaccine studies have usually been done with a virus inoculum size sufficient to infect all unimmunized control animals. Such an inoculum almost certainly contains a larger quantity of virus than is usually responsible for HIV-1 transmission between humans.

These nonhuman primate infections provide models of varying degrees of stringency for assessing HIV vaccine strategies. It has proven remarkably easy to protect chimpanzees from infection with the SF2 isolate of HIV-1, probably because the virus replicates so poorly in that species (*15*). Challenge studies with $HIV\text{-}1_{SF2}$ in chimpanzees are not likely to be sufficiently rigorous to provide useful data for predicting vaccine efficacy in humans. On the other hand, the SIV isolate Pbj14 replicates to such high titers in pig-tailed macaques during primary infection that vaccine-elicited immunity sufficient to prevent HIV-1 infection in humans may be incapable of preventing macaque infections with this virus (*22*). Although virus load in a number of SIV/macaque models appears to be comparable to that seen in HIV-1–infected humans, differences clearly exist in the biology of these virus-host interactions. The most striking of these differences is that macaques die sooner after infection with SIV than do humans infected with HIV-1. The rapidity and extent of $CD4^+$ lymphocyte loss in macaques infected with pathogenic SHIV isolates are greater than the $CD4^+$ cell loss induced by HIV-1 in humans (*21*). In fact, there is no perfect nonhuman primate model for predicting HIV-1 vaccine efficacy. However, the variety of models available affords an important opportunity to assess vaccine strategies in a number of different systems and thus gauge the potency of a particular vaccine-elicited immunity in preventing AIDS virus infections.

Live, Attenuated Vaccines

A live virus that has been genetically altered to attenuate its pathogenic potential can often elicit humoral and cellular immune responses that are comparable to those generated in naturally occurring wild-type virus infections. Such genetically altered viruses are currently used as vaccines to prevent polio, measles, and chicken pox in humans. Preclinical studies have been done to explore the potential utility of live, attenuated viruses for preventing HIV-1 infection. These studies received an early impetus from the report that infection of macaques with an SIV that was rendered nonpathogenic by deletion of the accessory gene *nef* protected these animals from detectable infection on subsequent challenge with pathogenic SIV (*23*). The initial suggestion that an accessory gene–deleted HIV-1 might provide a viable AIDS vaccine was met with considerable skepticism. Because HIV-1 mutates extremely rapidly, it was suggested that accumulating genetic alterations in a nonpathogenic virus used as a vaccine might lead eventually to the virus regaining its pathogenic potential. However, a cohort of Australian men and women, all of whom received contaminated blood

products that were traced to a single donor, were infected with a *nef*-deleted virus that does not appear to induce significant $CD4^+$ T cell loss and AIDS (*24*).

Experimental work has been invested in attempting to define the immune mechanisms that mediate the potent protection afforded by infection with a live, attenuated SIV. Immunoglobulin purified from serum of macaques that have been vaccinated with *nef*-deleted SIV has not protected naïve macaques from infection with pathogenic SIV (*25*). Rhesus monkeys infected with a *nef*-deleted SIV develop SIV-specific CTL responses (*26*). However, the adoptive transfer studies that would be needed to demonstrate that such CTLs are involved in this protective immunity cannot be performed in outbred populations of macaques. Prior infection with a *nef*-deleted SIV has been shown to protect macaques from infection not only with pathogenic SIVs, but with SHIVs that express an HIV-1 envelope (*27*). Although the interpretation of these findings must be tempered by the realization that some of the SHIVs used in these studies replicate only to low levels in macaques, this observation suggests that envelope-specific immunity may not be required for this protection. The immunity elicited by infection with a *nef*-deleted SIV may therefore be either CTL-based or may not be antigen-specific.

An AIDS vaccine must, in the end, be safe. There is accumulating evidence in macaques that the genetic strategies that have been used to date cannot create a safe live, attenuated AIDS virus vaccine (Table 2). A pathologically attenuated SIV isolate containing a 12-nucleotide deletion in *nef* regenerated a complete *nef* gene during the course of infection in macaques, thereby regaining its pathogenic potential (*28*). More troubling, however, were the recent reports that even SIVs with persistent accessory gene deletions are proving pathogenic in neonates and even in adult macaques that have been infected for a prolonged period of time (*29*). It remains an open question as to whether a genetically deleted AIDS virus can replicate to a high-enough level in vivo to interfere with infection by another AIDS virus isolate and still not induce disease during a prolonged period of infection.

Inactivated Viruses with Adjuvant

Inactivated whole viruses delivered with a variety of adjuvants can provide long-lasting protection in humans against a number of viruses, including influenza and polio virus. In spite of fears that incomplete inactivation of HIV-1 might lead to inadvertent infection of vaccinees, considerable interest was generated for this strategy early in the AIDS epidemic by studies in which macaques immunized with inactivated SIV were readily protected against homologous pathogenic SIV challenges (*30*). However, enthusiasm for this vaccine approach was substantially dampened by subsequent findings suggesting that the protective immunity generated in these immunized monkeys was not virus-specific. Rather, the protection seen in these vaccinated animals correlated with the presence of antibodies specific for the cells in which the vaccine virus was cultivated (*31*). Some degree of protection was even achieved by immunizing macaques with uninfected cells.

These cell-specific immune responses could have been elicited by cell-surface molecules in the vaccine preparation that either copurifed with the virus or were incorporated into the lipid bilayer of the virions as they budded from the membrane of infected cells. Antibodies to cell-surface molecules may interfere with infection either by binding directly to cellular proteins incorporated into the virions or by binding to the cell surface and blocking interactions between virus and receptors. A recognition that an HIV-1 vaccine cannot be based on antibodies to normal cell surface molecules rather than virus-specific antigenic determinants has led investigators to abandon this vaccine strategy.

Antigens that include viral proteins that maintain a tertiary conformation similar to that of the native virus remain attractive as vaccine immunogens. Selected HIV-1 proteins that are expressed in a cell line can, under certain conditions, self-assemble into particles (*32*). If these expressed viral proteins do not include reverse transcriptase, these particles are not replication competent. Although there is interest in such viruslike particles as immunogens, the technical problem of maintaining intact envelope in such vaccine preparations after virus purification has thus far limited their utility.

Subunit Vaccines

Highly purified viral proteins can be produced relatively inexpensively by expression of viral genes in tumor cell lines or yeast. A recombinant protein made in this manner is used in the successful hepatitis B vaccine. Considerable effort has been directed toward evaluating recombinant monomeric HIV-1 envelope proteins as vaccine immunogens. A limited number of chimpanzee studies have suggested that this approach can protect against an intravenous cell-free HIV-1 challenge (*33*). However, these studies were done with laboratory-adapted challenge viruses which, in most instances, did not replicate to very high levels in the chimpanzees. Proteins of this type have been assessed for immunogenicity in human studies using a variety of novel adjuvant formulations. These studies uniformly demonstrated that this vaccine strategy does not elicit HIV-1 envelope-specific CTLs and does not generate antibody responses that can neutralize primary patient isolates of HIV-1 (*34*). Moreover, in immunogenicity trials in high-risk human populations in the United States, infections of multiply immunized volunteers have been reported in which the vaccine-elicited immunity exerted no selective pressure on the infecting virus and had no effect on the clinical outcome of the infections (*35*). Although the National Institutes of Health chose not to proceed with large-scale efficacy trials of a recombinant HIV-1 envelope vaccine, other U.S. government agencies and private vaccine manufacturers are proceeding with trials of this vaccine strategy in Thailand with a bivalent immunogen that includes an envelope sequence typical of the clade E viruses commonly found in that geographic region.

Table 2. Current strategies for an HIV-1 vaccine.

Approach	Limitations
Live, attenuated viruses	Eventual pathogenicity in vaccinees
Inactivated viruses with adjuvants	Protection based on anti-cell rather than antiviral antibodies
Subunit vaccines	
Recombinant monomeric envelope protein	Absence of antibodies that neutralize patient isolates of HIV-1; absence of CTLs
Peptides	Absence of antibodies that neutralize patient isolates of HIV-1
Live vector-based vaccines (pox viruses, single-strand RNA viruses, adenovirus, bacille Calmette-Guérin, and enteric bacteria)	Immunogenicity, and level of in vivo replication and pathogenicity of vector closely correlated
DNA plasmids	Little experience with approach

Our growing understanding of envelope as it is expressed by primary patient HIV-1 isolates suggests that the use of recombinant envelope glycoproteins as immunogens bears revisiting. It has become clear that tissue culture adaptation of HIV-1 alters the configuration of the envelope glycoprotein, exposing its third hypervariable (V3) loop as a neutralization determinant in a manner that does not occur in patient isolates of the virus (*36*). Recent data also suggest that the native envelope exists as a trimer rather than a monomer (*37*). Furthermore, the conformation of the HIV-1 envelope clearly changes as it associates with CD4 and a chemokine receptor (9). Recombinant envelope glycoprotein immunogens that are based on primary patient isolate envelope sequences expressed as oligomers and that are presented to the immune system with an appreciation for the importance of their tertiary conformation, bear careful study. A recombinant protein immunogen, formulated in any available adjuvant, cannot efficiently elicit a CTL response. Such immunogens may, however, prove useful for eliciting neutralizing antibodies and prove efficacious in combination with other vaccine strategies.

Early enthusiasm for exploring peptide vaccines for preventing HIV-1 infection was based on the supposition that the V3 loop of HIV-1 was the principal neutralizing determinant of the virus and the demonstration that this determinant could be mimicked antigenically by synthetic peptides (*38*). Furthermore, adjuvanted peptides were shown to be capable of eliciting virus-specific CTL responses in a variety of experimental animals. However, lipopeptide formulations proved disappointing in their ability to elicit CTLs in limited human trials. Peptide vaccine strategies lost further favor when it became clear that the V3 loop is of less importance as a neutralizing determinant on patient isolates of HIV-1 than it is on laboratory-adapted virus isolates (9). Recent observations suggesting that the V3 loop may be important in gp120-CD4-chemokine receptor interactions (39), coupled with the attractiveness of using combinations of peptides as a strategy for overcoming the problem of sequence diversity among HIV-1 isolates, and their ability as immunogens to elicit CTLs, leave peptides with a potential role in combination modality approaches to an HIV-1 vaccine.

Live Vector-Based Vaccines

Genes encoding viral proteins can be inserted into the genomes of other viruses or bacteria. The resulting recombinant organisms then express the products of the inserted genes. Infection of an experimental animal or human with such recombinant viruses or bacteria leads to immune responses to the parental organisms and to the products of the inserted genes. Vaccines based on this recombinant approach have the potential to elicit immunity of the magnitude and duration generated through an infection but without pathogenic consequences. The utility of this vaccine approach is, however, constrained by the size of the gene that can be carried by the parental vector and the in vivo replicative capacity, genetic stability, and pathogenicity of the recombinant organism.

The best-studied vaccine vectors are the pox viruses. Recombinant organisms created by inserting AIDS virus genes into vaccinia virus (the live, attenuated vaccine virus that has eliminated smallpox infections worldwide) have elicited AIDS virus–specific cellular and humoral immunity in macaques (*40*). Moreover, when immunizations with such constructs have been followed with boosting by recombinant proteins, vaccinated monkeys have been protected against infection by some SIV isolates (*41*). These findings suggest that vaccinia virus should be an extremely promising vector for use as an HIV vaccine. However, humans immunosuppressed as a consequence of HIV infection who have been inoculated with vaccinia have developed life-threatening disseminated vaccinia infections (*42*). In view of the large numbers of immunosuppressed, HIV-infected individuals in regions of the world where an HIV vaccine must be administered, and the possibility of spread of vaccinia from vaccinees to these susceptible individuals, there is a reluctance to use this vector system in large-scale human trials.

Interest has, therefore, turned to pox viruses with limited in vivo replicative capacity and, therefore, limited pathogenic potential in humans. A vaccinia strain that was multiply passaged in vitro, modified vaccinia ankara (MVA), has deletions of a number of genes associated with its pathogenicity (*43*). When MVA was used as a vaccine vector for SIV genes, immunization before SIV infection substantially delayed the clinical progression of AIDS in half of the immunized macaques (*44*). Although expanded studies of this pox vector for use as an HIV-1 vaccine are ongoing in nonhuman primates, the absence of a proprietary position on this technology has slowed the progress of its development for testing in humans.

Avian pox viruses do not complete an entire replication cycle in human cells. However, they initiate protein synthesis and, in so doing, can elicit an immune response. In view of the excellent safety profile of the avian pox viruses in humans, recombinant HIV-1 vaccines created with these vectors have undergone extensive preclinical and early-phase human testing. Studies have been done on more than 300 human volunteers who were immunized with canary pox vectors encoding both HIV-1 envelope and gag in a variety of dosages and vaccination schedules. Although the responses in these individuals varied as a result of the particular immunization protocol, low-titer envelope- and gag-specific antibodies were detected in 70% of vaccinees (*45*). During the course of repeated vaccinations with both envelope- and gag-expressing canary pox constructs, at any single sampling time, 29% of individuals have either an envelope- or gag-specific CTL response. Throughout the entire course of study, at least a single positive CTL response to either envelope or gag has been detected in 53% of vaccinees (*46*). Many of these responses have, however, not proven durable, with some subjects demonstrating only sporadically detectable CTL responses. Although the consistency of HIV-1-specific CTL elicitation was certainly not optimal, this vaccine approach does elicit effector T cell immunity. Whether this degree of immunogenicity is sufficient to warrant testing of this vector strategy in large-scale human efficacy trials will be debated by scientists in the coming months.

Other viruses that are being evaluated as vaccine vectors in preclinical studies include adenovirus and a number of single-strand RNA viruses. Because of the presumed importance of mucosal immunity in protecting against an HIV-1 infection and the ability of adenoviruses to elicit mucosal immune responses, adenoviruses would appear to be attractive vectors for inducing HIV-1–specific immunity. Although the immunity generated by recombinant serotype 5 and 7 adenoviruses has not proven very effective, work continues in this area with some of the more immunogenic gene-deleted adenoviruses that have been developed as gene therapy vectors (*47*). Investigators are just beginning to evaluate the potential utility of polio virus and the alpha viruses (including Semliki forest virus and Venezuelan equine encephalitis virus) as HIV vaccine vectors (*22*, *48*).

Some effort has also gone into the evaluation of recombinant bacteria as HIV vaccine vectors. The pathologically attenuated bacterium that is used to vaccinate against *Mycobacterium tuberculosis* infection, bacille Calmette-Guérin (BCG), is attractive as a vaccine vector candidate because it establishes a chronic, persistent infection and has proven to be safe in worldwide use. Limited studies in nonhuman primates have indicated that infection of rhesus monkeys

with recombinant BCG can elicit AIDS virus–specific CTL responses (*49*). Enteric bacteria, including salmonella and shigella, have also generated interest because of their ability to elicit mucosal immune responses. The recombinant enteric bacteria studied to date, however, have not proven particularly immunogenic.

The live vector approaches that have been evaluated have, in general, shared a single shortcoming. The immunogenicity of a vector is closely tied to the extent of replication that vector undergoes in vivo, and the pathogenicity of a vector is similarly correlated with the extent of in vivo replication of that organism. Therefore, the most immunogenic of the live vectors have also proven to be the most pathogenic. Nevertheless, these strategies remain attractive and will be intensively explored in the coming years.

DNA Vaccines

Direct injection of plasmid DNA expressing a gene encoding the protein of a pathogen has proven to be an effective vaccination modality (*50*). After intramuscular injection or intradermal introduction by means of a gene gun, DNA vaccine plasmids are taken up by cells and the encoded protein antigens are expressed. The proteins are processed, and strong and persistent humoral and cellular immune responses are generated. Immune responses elicited in this way confer protective immunity against influenza challenge in mice and ferrets. DNA immunization offers the advantage of eliciting antibody and CTL responses without the pathogenic risks inherent in immunization with live vectors.

A number of reports have shown that DNA vaccines can generate HIV- and SIV-specific CTL, helper T cells, and antibodies in mice and nonhuman primates. In addition, several plasmid DNA vaccine trials in nonhuman primates involving viral challenges have shown protective immunity (*51*). However, these chimpanzee and macaque studies have demonstrated protection against AIDS viruses that replicate only to low levels. The optimal use of this type of vaccine and its ultimate potential for blocking HIV-1 infections should be clarified in the near future.

Therapeutic Vaccines

Before highly active antiretroviral therapies (HAART) became available, a number of strategies were pursued to assess the utility of vaccines as therapies for HIV-1–infected individuals. The rationale for such an approach was the assumption that biologically useful immune responses to HIV-1 that do not occur as part of the normal host response might be generated through vaccination. Large studies done in chronically infected individuals with recombinant envelope protein and with inactivated virion immunogens failed to demonstrate a convincing antiviral effect (*52*). With the advent of HAART, a new rationale has been proposed for exploring vaccine therapies in infected individuals who have responded to antiviral drugs. Treated individuals frequently show waning humoral and cellular immune responses to HIV-1 as their load of viral antigen decreases (*53*). It has been suggested that vaccine-elicited immunity in such individuals may result in even greater containment of viral replication, perhaps eventually allowing withdrawal of HAART. Studies to assess this possibility will be pursued by a number of laboratories.

Defining a Successful Vaccine Strategy

A number of vaccine strategies have prevented infection of nonhuman primate species with AIDS viruses of low replication potential, but these same strategies have not prevented infections with viruses that replicate to high levels (*22*, *41*). Because HIV-1 usually maintains a high level of replication in humans, this finding has led some to suggest that an AIDS vaccine might never be able to generate sterilizing immunity against primary patient isolates of HIV-1. It has been proposed that a realistic goal for a vaccine should, therefore, be to generate immunity that will change the long-term virologic and clinical consequences of HIV-1 infection: damping viral replication during primary infection, lowering the steady state of viral replication during chronic infection, and slowing the progression of clinical disease. In fact, in many nonhuman primate vaccine trials in which sterilizing immunity has not been achieved, a number of these changes in the clinical sequelae of infection have been demonstrated.

Vaccine-elicited immunity generated before HIV-1 infection may decrease viral replication sufficiently in a vaccinee to alter the clinical sequelae of infection and diminish the likelihood of that individual subsequently transmitting HIV-1 to others. If this proves to be the case, even an imperfect HIV-1 vaccine might result in prolonged survival for those subsequently infected and a slowing of virus spread to uninfected individuals. Yet testing the ability of a vaccine to slow disease progression in infected vaccinees in this era of readily available, highly effective antiviral therapies is extremely problematic. Accruing data strongly suggest that the earliest possible treatment of infected individuals is desirable (*54*). Assessing the impact of prior vaccination on the clinical disease course after HIV-1 infection could, therefore, require withholding appropriate, available therapies.

There is today, however, reason to persist in efforts to generate sterilizing immunity to HIV-1. Protection against AIDS virus infections has been achieved in nonhuman primates with a variety of strategies. We should be able to increase the efficiency of vaccines to elicit the HIV-1–specific CTL and neutralizing antibody responses that mediate this protection through the application of currently available technologies. Moreover, our growing understanding of the biology of HIV-1 and immune responses to this virus will continue to suggest improved vaccination approaches for exploration.

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HIV-1 Regulatory/Accessory Genes: Keys to Unraveling Viral and Host Cell Biology

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Human immunodeficiency virus type–1 (HIV-1) manipulates fundamental host cell processes in sophisticated ways to achieve optimum replicative efficiency. Recent studies have provided new details on the molecular interactions of HIV-1 with its host cell. For example, HIV-1 encodes a protein that regulates transcriptional elongation by interacting with a cellular cyclin-dependent kinase, another that activates the specific nuclear export of viral RNA, and several others that affect the intracellular trafficking of viral and host cell proteins. Detailed analysis of the interplay between these viral proteins and normal cellular activities has provided new insights into central questions of virology and host cell biology.

HIV-1 is a member of one of the five major primate lineages of the lentivirus family of retroviruses (*1*). Although the basic steps of the HIV-1 life cycle are the same as for other retroviruses, six virally encoded regulatory/accessory proteins (Tat, Rev, Vif, Vpr, Vpu, and Nef) that are not found in other classes of retroviruses impart novel levels of complexity to lentiviral replication (*2*). Here we review some of the most recent progress in our understanding of the interactions between these gene products and host cell factors and discuss possible selective pressures that have imposed the need for these specialized viral proteins.

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Transcriptional Control by Manipulation of Host Cell Factors

High-level HIV-1 transcription from the integrated DNA form of the virus (the provirus) is regulated by an ~14-kD viral protein called Tat. The domain structure of Tat is typical of many transcriptional activators and includes an activation domain and a nucleic acid (in this case, RNA) binding domain. Tat function is dependent on a bulged RNA stem-loop structure, TAR (Tat activation region), that is present at the 5′-terminus of all viral mRNAs (Fig. 1). Although HIV-1 transcription is mediated by cellular RNA polymerase II, Tat acts mostly at the level of transcriptional elongation rather than at initiation itself. Because of this apparently novel mode of transcriptional regulation, there has been a prolonged effort to identify cellular Tat cofactors (*3*). It was anticipated that definition of a cellular cofactor or cofactors could explain several intriguing observations about Tat trans activation: First, binding of recombinant Tat to TAR in vitro does not require the loop sequences known to be necessary in vivo for function; second, preincubation of nuclear extracts with the activation domain of recombinant Tat depletes a factor necessary for Tat-mediated transcription in vitro; and finally, Tat functions poorly in rodent cells unless complemented by a factor or factors that can be supplied in trans by human chromosome 12. A cellular protein complex whose attributes explain these diverse phenomena has now been found.

A cellular protein kinase complex called TAK (Tat-associated kinase) was identified that specifically binds to the activation domain of Tat and can phosphorylate the COOH-terminal domain (CTD) of RNA polymerase II (*4*)—a step that had already been implicated in regulation of transcriptional elongation (*5*). The kinase component of TAK was then shown to be the same as a previously identified kinase named PITALRE that was also implicated in transcriptional elongation. The kinase activity of the PITALRE complex is disrupted by compounds that were identified during an in vitro drug screen as inhibitors of Tat activity (*6*). PITALRE has since been renamed Cdk9 because it is related to the family of cyclin-dependent kinases (Cdks).

By analogy with other Cdks, it was assumed that Cdk9 would have a cyclin-related partner that would confer substrate specificity on the kinase. This protein has been identified and is called cyclin T (CycT) (*7*). CycT binds the activation domain of Tat both on its own and in the context of a Cdk9-CycT complex (Cdk9 does not bind Tat on its own) (Fig. 1). CycT increases the affinity of Tat for TAR, increases the specificity of Tat for residues of TAR known to be important for activity (the loop and bulge residues), and is necessary for transcriptional